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AMENDMENTS
In the Specification

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Suitable atomic tag for use in this invention include, without limitation, any atomic element amenable to att,achment to a specific site in a polymerizing agent or dNTP, especially fluorescent dyes such as d-Rhodamine acceptor dyes including dichloro[R110], dichloro[R6G], dichloro[TAMRA], dichloro[ROX] or the like, fluorescein donor dye including fluorescein, 6-FAM, or the like;Acridine including Acridine orange, Acridine yellow, Proflavin, pH 7, or the like; Aromatic Hydrocarbon including 2-Methylbenzoxazole, Ethyl p-dimethylaminobenzoate, Phenol, Pyrrole, benzene, toluene, or the like; Arylmethine Dyes including Auramine O, Crystal violet, H2O, Crystal violet, glycerol, Malachite Green or the like; Coumarin dyes including 7-Methoxycoumarin-4-acetic acid, Coumarin 1, Coumarin 30, Coumarin 314, Coumarin 343, Coumarin 6 or the like; Cyanine Dye including 1,1'-diethyl-2,2'-cyanine iodide, Cryptocyanine, Indocarbocyanine (C3)dye, Indodicarbocyanine (C5)dye, Indotricarbocyanine (C7)dye, Oxacarbocyanine (C3)dye, Oxadicarbocyanine (C5)dye, Oxatricarbocyanine (C7)dye, Pinacyanol iodide, Stains all, Thiacarbocyanine (C3)dye, ethanol, Thiacarbocyanine (C3)dye, n-propanol, Thiadicarbocyanine (C5)dye, Thiatricarbocyanine (C7)dye, or the like; Dipyrrin dyes including N,N'-Difluoroboryl-1,9-dimethyl-5-(4-iodophenyl)-dipyrrin, N,N'-Difluoroboryl-1,9-dimethyl-5-[(4-(2-trimethylsilylethynyl), N,N'-Difluoroboryl-1,9-dimethyl-5-phenydipyrrin, or the like; Merocyanines including 4-(dicyanomethylene)-2-methyl-6-(p-dimethylaminostyryl)-4H-pyran (DCM), acetonitrile, 4-(dicyanomethylene)-2-methyl-6-(p-dimethylaminostyryl)-4H-pyran (DCM), methanol, 4-Dimethylamino-4'-nitrostilbene, Merocyanine 540, or the like; Miscellaneous Dye including 4',6-Diamidino-2-phenylindole (DAPI), 4',6-Diamidino-2-phenylindole (DAPI), dimethylsulfoxide, 7-Benzylamino-4-nitrobenz-2-oxa-1,3-diazole, Dansyl glycine, H2O, Dansyl glycine, dioxane, ~~Hoechst~~ HOECHST® 33258, DMF, ~~Hoechst~~ HOECHST® 33258, H2O, Lucifer yellow CH, Piroxicam, Quinine sulfate, 0.05 M H2SO4, Quinine sulfate, 0.5 M H2SO4, Squarylium dye III, or the like; Oligophenylenes including 2,5-Diphenyloxazole (PPO), Biphenyl, POPOP, p-Quaterphenyl, p-Terphenyl, or the like; Oxazines including Cresyl violet perchlorate, Nile Blue, methanol, Nile Red, Nile blue, ethanol, Oxazine 1, Oxazine 170, or the like; Polycyclic Aromatic Hydrocarbons including 9,10-Bis(phenylethynyl)anthracene, 9,10-Diphenylanthracene, Anthracene, Naphthalene, Perylene, Pyrene, or the like; polyene/polyynes including 1,2-diphenylacetylene, 1,4-diphenylbutadiene, 1,4-

diphenylbutadiyne, 1,6-Diphenylhexatriene, Beta-carotene, Stilbene, or the like; Redox-active Chromophores including Anthraquinone, Azobenzene, Benzoquinone, Ferrocene, Riboflavin, Tris(2,2'-bipyridyl)ruthenium(II), Tetrapyrrole, Bilirubin, Chlorophyll a, diethyl ether, Chlorophyll a, methanol, Chlorophyll b, Diprotonated-tetraphenylporphyrin, Hematin, Magnesium octaethylporphyrin, Magnesium octaethylporphyrin (MgOEP), Magnesium phthalocyanine (MgPc), PrOH, Magnesium phthalocyanine (MgPc), pyridine, Magnesium tetramesitylporphyrin (MgTMP), Magnesium tetraphenylporphyrin (MgTPP), Octaethylporphyrin, Phthalocyanine (Pc), Porphin, Tetra-t-butylazaporphine, Tetra-t-butylnaphthalocyanine, Tetrakis(2,6-dichlorophenyl)porphyrin, Tetrakis(o-aminophenyl)porphyrin, Tetramesitylporphyrin (TMP), Tetraphenylporphyrin (TPP), Vitamin B12, Zinc octaethylporphyrin (ZnOEP), Zinc phthalocyanine (ZnPc), pyridine, Zinc tetramesitylporphyrin (ZnTMP), Zinc tetramesitylporphyrin radical cation, Zinc tetraphenylporphyrin (ZnTPP), or the like; Xanthenes including Eosin Y, Fluorescein, basic ethanol, Fluorescein, ethanol, Rhodamine 123, Rhodamine 6G, Rhodamine B, Rose bengal, Sulforhodamine 101, or the like; or mixtures or combination thereof or synthetic derivatives thereof or FRET fluorophore-quencher pairs including DLO-FB1 (5'-FAM/3'-BHQ-1) DLO-TEB1 (5'-TET/3'-BHQ-1), DLO-JB1 (5'-JOE/3'-BHQ-1), DLO-HB1 (5'-HEX/3'-BHQ-1), DLO-C3B2 (5'-Cy3/3'-BHQ-2), DLO-TAB2 (5'-TAMRA/3'-BHQ-2), DLO-RB2 (5'-ROX/3'-BHQ-2), DLO-C5B3 (5'-Cy5/3'-BHQ-3), DLO-C55B3 (5'-Cy5.5/3'-BHQ-3), MBO-FB1 (5'-FAM/3'-BHQ-1), MBO-TEB1 (5'-TET/3'-BHQ-1), MBO-JB1 (5'-JOE/3'-BHQ-1), MBO-HB1 (5'-HEX/3'-BHQ-1), MBO-C3B2 (5'-Cy3/3'-BHQ-2), MBO-TAB2 (5'-TAMRA/3'-BHQ-2), MBO-RB2 (5'-ROX/3'-BHQ-2); MBO-C5B3 (5'-Cy5/3'-BHQ-3), MBO-C55B3 (5'-Cy5.5/3'-BHQ-3) or similar FRET pairs available from Biosearch Technologies, Inc. of Novato, CA, tags with nmr active groups, tags with spectral features that can be easily identified such as IR, far IR, visible UV, far UV or the like.

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EXAMPLES

Cloning and Mutagenesis of *Taq* Polymerase

Cloning

Bacteriophage lambda host strain Charon 35 harboring the full-length of the *Thermus aquaticus* gene encoding DNA polymerase I (*Taq* pol I) was obtained from the ~~American Type Culture Collection~~ AMERICAN TYPE CULTURE COLLECTION (ATCC; Manassas, VA). *Taq* pol I was amplified directly from the lysate of the infected *E. coli* host using the following DNA

oligonucleotide primers:

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1-Aminonaphthalene-5-sulphonic acid (447 mg, 2 mmol, 40 eq., from Lancaster) was added to 10 mL of H_2O, and the pH was adjusted to 5.8 with 1 N NaOH. The insoluble material was removed by syringe filter, yielding a solution which was essentially saturated for this pH value (~0.18 to 0.2 M). 4 mL of 12.5 mM 5'triphosphate-2'-deoxyadenosine disodium salt (0.05 mmol, 1 eq.) and 2 mL of 1 M 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (DEC, 2 mmol, 40eq., from Lancaster) were added to a reaction vessel at 22 °C. The reaction was initiated by adding 10 mL of the 1-aminonaphthalene-5-sulfonate solution and allowed to continue for 2.5 h. The pH was kept between 5.65 - 5.75 by periodic addition of 0.1 N HCl. After 2.5 h, the reaction was diluted to 50 mL and adjusted to a solution of 0.05 M triethylammonium bicarbonate buffer (TEAB, pH ~7.5). The reaction product was chromatographed on a 50 mL DEAE-SEPHADEX® ion exchanger (A-25-120) column at low temperature that was equilibrated with ~pH 7.5 1.0 M aqueous TEAB (100 mL), 1.0 M aqueous sodium bicarbonate (100 mL), and ~pH 7.5, 0.05 M aqueous TEAB (100 mL). The column was eluted with a linear gradient of ~pH 7.5 aqueous TEAB from 0.05 to 0.9 M. Approximately 10 mL fractions were collected. Absorbance and fluorescence profiles (UV 366nm) of the fractions were obtained after appropriate dilution. The fluorescent fraction eluted at ~0.7 M buffer after the peak of the unreacted dATP and showed a brilliant blue fluorescence. The product-containing fractions were pooled, dried by lyophilizer and co-evaporated twice with H_2O/ethanol (70/30). The residue was taken up in water and lyophilized. ^{31}P NMR (^1H decoupled, 600 MHz, D_2O, Me_3PO4 external reference, 293 K, pH 6.1) δ (ppm) -12.60, -14.10, -25.79. The reference compound dATP gave the following resonance peaks: ^{31}P NMR (dATP, Na^+) in D_2O 293 K, δ (ppm) -11.53 (γ), -13.92 (α), -24.93 (β).

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The indicated enzyme (*Taq* DNA Polymerase, ~~Sequenase~~ SEQUENASE®, HIV-1 Reverse Transcriptase, T7 DNA Polymerase, Klenow Fragment, *Pfu* DNA Polymerase) were incubated in the manufacturers suggested reaction buffer, 50 μM of the indicated nucleotide at 37°C for 30 - 60 minutes, and the reaction products were analyzed by size separation through a 20% denaturing gel.

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Polymerization activity is difficult to detect in the reaction products generated by native T7 DNA polymerase (due to the presence of the enzymes exonuclease activity). However, its

genetically modified derivative, ~~Sequenase~~ SEQUENASE®, shows that the gamma-modified nucleotides are efficiently incorporated, and that incorporation fidelity is improved, relative to non-modified nucleotides. The experimental results for native T7 DNA polymerase and ~~Sequenase~~ SEQUENASE® are shown in Figure 2.

Thus, for the Taq polymerase or the HIV1 reverse transcriptase, improved fidelity, due to the use of the gamma-modified dNTPs of this invention, enables single-molecule DNA sequencing. However, not all polymerases equally utilize the gamma-modifed nucleotides of this invention, specifically, Klenow, ~~Sequenase~~ SEQUENASE®, HIV-1 reverse transcriptase and Taq polymerases incorporate the modifed nucleotides of this invention, while the *Pfu* DNA polymerase does not appear to incorporate the modified nucleotides of this invention.